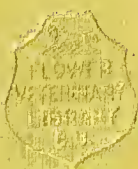


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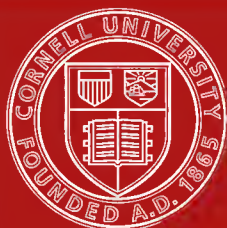
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FATAL SEPTICEMIA OR BACILLARY WHITE DIARRHEA IN YOUNG CHICKENS.

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Perhaps there is not a more difficult problem before the breeders of stock to-day than that confronted by the poultrymen in successfully caring for chickens during the first ten weeks of their lives. Until two years ago the heavy mortality among young chickens had not been investigated scientifically. Poultrymen generally regarded this loss as a result of indirect causes, such as defective heating of the incubators and brooders and improper feeding. The rapid increase of these troubles brought about investigations to ascertain the cause and to formulate, if possible, preventive measures for their control. The disease or condition which seems to have given the most trouble is that popularly known as "white diarrhea." The results of the different investigations of these disorders recently reported have suggested different causes that should be considered.

Up to the present the term "white diarrhea" has been found to represent four infectious diseases, as well as other morbid conditions that may cause death or sickness with a diarrhea. The four distinct diseases now embraced in the term "white diarrhea" have been classified under the following heads, namely: (1) coccidiosis, (2) fatal septicemia or bacillary white diarrhea, (3) aspergillosis or mycosis, and (4) enterohepatitis.

In February 1908, Morse published a paper entitled "White Diarrhea in Chicks with Notes on the Coccidiosis of Birds." In this paper he claims that a great part of the so-called white diarrhea is due to coccidiosis, identifying the parasite as *Coccidium tenellum*. It was also stated in this paper that the same class of parasites causes losses to duck, turkey and pigeon breeders.

In July 1908, Rettger and Harvey published a paper entitled "Fatal Septicemia in Young Chickens, or White Diarrhea," in which the authors stated that the cause of the disease with which they worked was a bacterium named by them *Bact. pullorum*.

In August 1908, Milks of the Louisiana Experiment Station confirmed the results of Rettger and Harvey. He also described infectious enterohepatitis, the cause of which he found to be *Amoeba melagrides*, the organism described by Smith as the cause of this disease in turkeys.

In October 1909, Cushing described a fourth form of white diarrhea in the *Canadian Poultry Review*, under the title of *Aspergillosis*. Although this is not by any means the first description of aspergillosis in birds, it seems to be the first time the disease has been described as a large epizootic among young chickens in America.

Our attention was first called to fatal septicemia in the spring of 1910. At that time a serious outbreak occurred among young chicks in which the losses were very heavy. On April 13, 1910, six chicks one week old were received at the laboratory. They had been dead about eighteen hours. Post-mortem examinations revealed the following: The heart normal in size but pale, the liver a dull ochre color and banded with stripes bright red in color, the spleen somewhat enlarged, softened and slate gray in color, the stomach, gizzard and intestines pale and containing a slimy fluid. A large unabsorbed yolk was found in each of the chicks; the blood vessels surrounding the yolk were congested. Culture media were inoculated from the heart blood, liver and unabsorbed yolk from each chick and in nearly every tube pure cultures of a bacterium developed which later was identified as *Bact. pullorum*.

Correspondence with the poultryman from whom the chicks were received revealed some very interesting facts. In the previous year (1909) he had lost 1,800 chicks and the conditions did not seem to be improved this year, for the mortality had been 90 per cent. in the incubator chicks and even higher in those hen hatched. On visiting the plant it was found to be clean and sanitary and managed in an up-to-date manner. The brooder house was clean, although it had not been disinfected so thoroughly or so often as necessary under the circumstances. Hot water heat was supplied to each hover. The adult birds were not confined but had practically free range. The percentage of fertility in the eggs was very high for heavy birds. It was impossible to obtain a clear history of the disease in the flock but the later hatches in 1908 suffered a high death rate although not so extensive as in 1909 and 1910. Learning that the poultryman had shipped day old chicks to purchasers in various parts of the country, it was decided to trace them and discover if possible whether any marked losses had occurred. The first lot had been shipped twenty-five miles and consisted of 104 chicks which were divided among several farmers; at the end of two weeks but thirty-eight of the original 104 remained well, the others having died from some

disease. Another purchaser received 100 and at the end of two weeks had lost fifty-five. Both of these parties received the chicks in good condition. They were removed from the incubators, boxed and expressed to the purchasers and had not been placed in the brooders of the infected plant. These facts seemed to indicate that the chicks were infected very early in life.

DESCRIPTION OF THE DISEASE.

Symptoms. The symptoms are not characteristic but are practically the same as in other chicken diseases. Probably the first symptom is the desire for more heat, staying under the hover and close to the heating surfaces. This is followed by marked depression, loss of appetite, labored breathing. The chick stands with feet far apart, eyes dull, head drawn down, and respirations are increased and labored. Diarrhea is usually present and in the cases of longer standing the feces gum up the tail feathers, often producing a mass as large as a walnut. This is what poultrymen call "Pasting up behind." In the cases of longer standing (1 to 3 weeks) emaciation becomes marked, the gait is uncertain, the legs seem to grow but the body remains practically the same size, causing the expression "short backed."

Post mortem lesions. The crop may or may not be filled with food. It is usually pale and it contains a slimy fluid.

The stomach and gizzard are pale and contain a slimy fluid.

The intestines are usually pale and slimy. The ceca are normal in size. The colon is often filled with feces due to the blocking up of the rectum.

The liver has a striped appearance, the background being a dull ochre color banded with stripes of bright red tissue. It is usually normal in size and very often is quite soft. The gall bladder in the acute cases is normal, but in the more chronic cases it is distended with dark colored bile.

The spleen is somewhat enlarged and a trifle soft, the capsule seems to separate from the pulp. The organ usually assumes a slate gray color.

The heart is usually pale and the ventricles are filled with dark coagulated blood.

The kidneys are usually pale and very soft, the ureters are filled with white urates.

The lungs are usually very much congested.

The unabsorbed yolk is the most characteristic lesion of the disease. The yolk varies in size from a full undigested yolk to the size of a pin head. The consistency, like the size, varies with the course of the disease; in the early cases it is large and watery while in those of longer standing it is small and more gelatinous in character. In the more chronic form it is hard and cheesy and somewhat difficult to cut. The blood vessels surrounding the yolk are highly congested.

In a large number of cases the umbilicus does not seem to heal properly and a partially healed opening remains which usually emits a bad odor.

“*The microscopic findings.*” Lung: There is congestion and sometimes hemorrhage into the bronchiols.

Liver: There is usually marked congestion, cloudy swelling and fatty degeneration. There are often small foci of necrosis. The specific bacteria are found, in properly stained preparations, in small numbers in the capillaries throughout the organ.

Kidney: Parenchymatous degeneration in the cortex and medulla, congestion in the capillaries between the tubules, fibrin in the tubules.

Spleen: The spleen is usually congested.

Intestines: There is usually a degeneration of the mucosa and almost no congestion.

Etiology: As already stated, ordinary media inoculated from the different organs developed pure cultures of *Bact. pullorum*. The biology of the organism is as follows:

Morphology: Rod shaped, varying in size from 2 to 3.5 microns in length and having an average width of .5 of a micron. The ends are rounded. Although active Brownian movement is usually observed, flagella could not be demonstrated. Capsules or spores have not been found. Usually the organisms are grouped singly or in pairs. They stain readily with all of the ordinary bacterial stains. They are Gram negative.

Agar colonies: At the end of forty-eight hours the colonies have reached their maximum development. At that time they are round, having a diameter of 1 to 2 mm. The surface is convex and the edges are entire. Under the low power they have an appearance of being finely granular. The growth is much denser in the center, giving it the appearance of a nucleus.

Slant agar: Cultures on this medium made directly from an infected individual develop a characteristic growth. The colonies

appear at the end of about twelve hours and continue to develop for forty-eight hours, when they appear as small flat droplets. They are round and rarely attain a greater diameter than 1 mm. They are raised above the surface of the medium. The surface is smooth, convex, glistening and of a grayish white color.

In the older cultures which have been transferred the growth resembles *B. typhosus* very closely. It is moderate and does not spread. It is glistening, smooth and translucent. The water of condensation becomes slightly cloudy and a heavy white sediment forms.

Gelatin: Growth is uniform, filiform and non-liquefying.

Potato: Does not grow on this medium.

Blood serum: The growth appears about the same as on agar.

Bouillon: Moderate clouding. Sediment is moderate and viscid on agitation. Reaction acid.

Milk: Not coagulated and becomes very slightly acid in forty-eight hours.

Glucose bouillon: Gas and acid or acid and no gas. Twenty-seven (27) per cent. of gas was produced, giving the following ratio: H 5: CO₂ 1. Three and one-half per cent. of acid was produced.

Lactose bouillon: Alkaline to litmus although when titrated five-tenths per cent. of acid was found to have been produced.

Saccharose bouillon: The medium remains alkaline.

Mannite bouillon: Acid or acid and gas, 65 per cent. of gas being formed having a ratio of H5:CO₂ 2. 3.9 per cent. of acid produced.

Glycerine bouillon: Alkaline.

Maltose bouillon: Alkaline.

The organism is easily killed by disinfectants, as the following table will show:

ACTION OF DISINFECTANTS.

Substance.	30 seconds.	1 min.	5 min.	10 min.	15 min.
1-1000 Hg Cl ²	Killed	Killed	Killed	Killed	Killed
1% Carbolic acid.....	Growth	Growth	Killed	Killed	Killed
1% Creolin.....	Growth	Growth	Killed	Killed	Killed
3 1-3% Lactic acid.....	Growth	Growth	Killed	Killed	Killed
5% Carbolic acid.....	Killed	Killed	Killed	Killed	Killed

Boiling killed the organisms in one minute. Heating to 60° C. for thirty minutes failed to kill them.

Pathogenesis: The organism is pathogenic to young chicks if inoculated orally within the first forty-eight hours after hatching. It is also pathogenic to chicks three days of age if injected subcutaneously. It causes death in from four days to three weeks and can be recovered from all organs. *Bact. pullorum* is not pathogenic to old fowls. Two cubic centimeters injected directly into the wing vein of adults produced marked depression which lasted for several days but no other bad effects were discovered. The same results were obtained when larger quantities were injected. No effect was produced by feeding large quantities of the culture to adults.

Half grown guinea pigs are susceptible when injected subcutaneously with the culture. They die in from twenty-four to forty-eight hours. The essential lesion is a large edematous area beneath the skin of the abdomen. The organisms are recovered in pure culture from all the internal organs. A rabbit weighing 1,660 grams was injected subcutaneously with 3 cc. of a forty-eight hour bouillon culture and seven days later died. The most pronounced lesions were enlargement, softening and congestion of the heart. The bacterium was recovered from all the internal organs in pure culture.

EXPERIMENTS.

In order to determine more definitely the pathogenesis of *Bact. pullorum*, the source of infection in young chicks and the means by which the disease is disseminated, a series of experiments were carried out. The experimental work was done in co-operation with the poultry department of the State College of Agriculture.

All of the experiments were carried out in a Paradise brooder containing six compartments. Before and after each experiment the brooders were thoroughly sprayed with one part carbolic acid and one part sulphuric acid to twenty parts of water. The chicks used were from the Leghorn experimental stock of the poultry department of the College of Agriculture. The parent birds were free from disease. The eggs were incubated in standard makes of incubators which were disinfected before using. All feed and grit were sterilized in the autoclave for forty-five minutes under thirteen pounds pressure. The water was boiled and the green food consisted of sprouted oats grown under sanitary precautions.

Experiment 1. This was for testing the pathogenesis of *Bact. pullorum*. May 5, 1910. Five chicks twenty-four hours old were each given orally 0.15 cc. of a 72-hour bouillon culture of the

organism isolated from the Canastota outbreak. One chick was given a subcutaneous injection of the same culture, the dose being 0.5 cc.

No. 795 died May 9. Pure cultures obtained from heart, liver, yolk.

No. 796 died May 8. Pure cultures obtained from heart, liver, yolk.

No. 797 died May 20. Pure cultures obtained from yolk.

No. 798 killed because it was too weak to stand, pure cultures obtained from the yolk.

No. 799 same as 798.

No. 789 (subcutaneous injection) died May 9. Pure cultures were obtained from the heart, liver and yolk.

Six chicks were kept in a separate pen but otherwise under the same conditions. None of them developed the disease.

This experiment was repeated May 31. The same culture was used as in the first experiment and each chick was given the same amount of the cultures. Two chicks forty-eight hours old were also fed the same quantity of the culture. These were numbered 11 and 12.

Chick No. 1 died June 5. Pure cultures obtained from yolk, liver and heart.

Chick No. 2 died June 5. Pure cultures obtained from yolk, heart, kidney and spleen, mixed in liver and lungs.

Chick No. 3 died June 5. Pure cultures obtained from liver and heart, mixed in yolk.

Chick No. 4 died June 6. Pure cultures obtained from liver and heart, mixed in yolk.

Chick No. 5 died June 6. Pure cultures obtained from liver and heart, mixed in yolk.

Chick No. 6 died June 10. Pure cultures obtained from liver and heart, mixed in yolk.

Chick No. 7 died June 11. Pure cultures obtained from liver, heart sterile, mixed in yolk.

Chick No. 8 died June 11. Pure cultures obtained from liver and heart, mixed in yolk.

Chick No. 9 died June 15. Pure cultures obtained from liver and heart, mixed in yolk.

Chick No. 10 died June 16. Mixed cultures obtained from heart, liver and yolk.

Chicks 11 and 12 were killed June 22, as they had shown marked symptoms and seemed to be chronic cases of the disease. The post-mortem showed the same general lesions as in the other chicks except that the yolk was absorbed. In No. 11 *Bact. pullorum* were obtained in mixed cultures from the liver and heart and in No. 12 pure cultures were obtained from the heart and mixed from the liver.

This experiment showed that (1) cultures of *Bact. pullorum* are pathogenic for chicks infected within the first twenty-four hours, (2) that the disease was a true septicemia, the organism being found in the heart, liver, lungs, spleen, kidney and yolk, and (3) that the period of incubation varied from two to five days. It is interesting to notice that the unabsorbed yolk was a somewhat constant lesion of the disease.

Experiment 2. May 31, 1910. This experiment consisted in placing chicks in the brooder used in the previous experiment, which was not disinfected or disturbed in any way. In this brooder fourteen twenty-four hour chicks and four forty-eight hour chicks were placed.

No. 1 died June 7. Post-mortem revealed the following: Crop empty and pale, stomach and gizzard pale and containing a slimy fluid; intestines pale with a slimy fluid content; liver dull ochre in color and striped with bright red bands; spleen somewhat enlarged and soft; heart pale, otherwise normal. An unabsorbed yolk was present and the blood vessels surrounding it very much congested. Inoculations from the heart blood, liver and yolk revealed the organism in pure culture.

No. 2 died June 11 (forty-eight hour chick). Unable to find the organism.

No. 3 died June 13. Pure cultures obtained from heart and liver and mixed in yolk.

No. 4 died June 16. Pure cultures obtained from heart and liver, yolk absorbed.

No. 5 died June 20. Pure cultures obtained from heart, mixed in liver, yolk absorbed.

No. 6 died June 22 (forty-eight hour chick). Pure culture obtained from heart, mixed in liver, yolk absorbed.

No. 7 died June 23. Pure culture obtained from liver and yolk.

No. 8 died June 25 (forty-eight hour chick). Mixed in heart and liver, yolk absorbed.

No. 9 died June 27. Pure in liver, heart sterile, yolk absorbed.

The remainder were killed on June 27 and inoculations were made from the organs of each.

No. 10. Pure cultures obtained from liver and heart, yolk absorbed.

No. 11. Pure cultures obtained from liver, heart sterile, yolk absorbed.

No. 12 (forty-eight hour chick). Heart and liver sterile, yolk absorbed.

No. 13. Heart and liver sterile, yolk absorbed.

No. 14. Pure cultures obtained from heart and liver, yolk sterile.

No. 15. All organs sterile.

No. 16. Pure cultures obtained from liver, heart sterile, yolk absorbed.

No. 17. All organs sterile.

No. 18. Mislaid, too putrid for examination.

This experiment showed that chicks may become infected by being placed in contaminated quarters. Twelve of the eighteen chicks used in the experiment became infected and the organism was recovered from the internal organs of each. At the end of three weeks the mortality was $44 \frac{4}{9}$ per cent. The experiment proves conclusively the necessity for disinfecting the brooders and utensils before placing a new hatch of chicks in them. After this experiment was completed the pen was thoroughly cleaned and disinfected and twenty-four hour chicks placed in it. The disease did not develop in any of these.

Experiment 3. This experiment consisted in the bacterial examination of eggs from poultry farms on which fatal septicemia had been found. Three shipments of eggs were received for examination. The first was from Canastota and consisted of twenty eggs. The second shipment was received from Hartwick Seminary and consisted of thirty-six eggs. The third lot of eggs was from Locke and numbered thirteen.

The eggs received from Canastota were divided into three groups: (1) The first group consisted of four eggs that were examined as soon as received. Slant agar tubes were inoculated from the scrapings of the outside of the shell and from the white and yolk. Growth occurred in nearly every tube but *Bact. pul-lorum* was not found.

(2) The second group consisted of six eggs that were incubated in a bacterial incubator for seventy-two hours at 38 degrees C.

Inoculations on slant agar were then made from the shell, white and yolk. The tubes remained sterile.

(3) The remaining twelve eggs were incubated for the usual time. Ten of the eggs hatched and only one of the chicks died within the first month. Cultures from the heart, liver and lung of this chick failed to develop *Bact. pullorum*. The two eggs which failed to hatch were found to be infertile. Cultures on slant agar from the white and yolk gave negative results.

Cultures on slant agar were made from the shells of all eggs received from Hartwick Seminary. A number of the tubes developed growths but *Bact. pullorum* was not found in any of these cultures. The eggs were then divided into three groups for separate examination. (1) Consisted of six eggs examined in a fresh condition. (2) Consisted of fifteen eggs that were dipped in a solution of 1-1000 corrosive sublimate and dried immediately. They were then incubated in full time. (3) The remaining fifteen eggs were then placed in an incubator without dipping.

(1) "The eggs examined fresh." Inoculations on slant agar were made from the white and yolk of each egg. Growth occurred in nearly every tube but *Bact. pullorum* was not found.

(2) "Eggs dipped in the corrosive sublimate solution." Eight of the fifteen eggs hatched on July 4. These chicks were placed in a pen which had been thoroughly disinfected before using. They were fed sterile feed and water. At the end of three weeks the first chick died of "leg weakness." Several of the others died from the same cause. Slant agar tubes were inoculated from the heart blood, liver and lung of each. All tubes remained sterile. On August 4 the remainder were killed and slant agar inoculations were made from the heart blood, liver and lung of each. The tubes remained sterile. The seven eggs that failed to hatch were examined bacteriologically. Inoculations were made on slant agar from the white and yolk of each egg. Not a tube developed *Bact. pullorum*.

(3) "Eggs incubated without dipping." Eight of these fifteen eggs hatched. At the end of one month none of the chicks had shown symptoms of the disease, although several had died from "leg weakness." The remainder were killed August 4 and examined bacteriologically for the presence of *Bact. pullorum* with negative results. The seven eggs that failed to hatch were examined bacteriologically. Inoculations upon slant agar were made from the yolk, the embryo and the white. In one egg *Bact. pul-*

lorum was found in pure culture. This egg contained a twenty-day embryo. The organism was found in great numbers in pure culture from the yolk of the embryo, the white surrounding it and the inside membrane of the shell.

The eggs received from Locke were incubated full time. Four of them hatched and none of these chicks developed the disease. The eggs that failed to hatch were examined bacteriologically for the presence of the organism but it was not found.

This experiment shows that *Bact. pullorum* may be present in the egg. The organism was not only found in the yolk of the embryo but in the white surrounding it and also on the inside membrane of the shell. It would be an easy matter for chicks coming in contact with these infected individuals and picking at pieces of the shell that contain the organisms to become inoculated with the virus. In this manner it would be a comparatively simple matter for a whole hatch of chicks to become infected before they left the incubator. The experiment further suggests that the number of eggs containing the organisms is not great, for but one of sixty-nine eggs obtained from the flocks of poultrymen who had recently lost chicks from the disease showed the presence of the specific organism.

Experiment 4. This experiment consisted in the inoculation of eggs with pure culture of *Bact. pullorum*.

Technique. The eggs were first dipped in a 1-1000 solution of corrosive sublimate and dried immediately. The large end of the egg was held uppermost to allow the yolk to rise so that it would not be punctured with the hypodermic needle during inoculation. A hole was then made in the small end of the egg. A small quantity of a forty-eight hour bouillon culture diluted in ten parts of sterile bouillon was then injected into the contents of the egg by means of a hypodermic syringe. Each egg received 0.1 cc. of the diluted culture. The hole was then sealed with collodion and cotton. The eggs were then incubated full time.

July 5, 1910. Lot A. Twenty eggs were inoculated with a pure culture of *Bact. pullorum* diluted one to ten. At the end of three weeks all failed to hatch. By candling the eggs it was discovered that the embryos had developed up to about the fifteenth day, when they had died. Inoculations from these eggs on slant agar revealed the organism in pure culture and in great numbers. It was present in every egg.

Lot B (check). Twenty eggs were inoculated with sterile bouillon and incubated in the same incubator with those of Lot A. At the end of three weeks fifteen of the eggs hatched, two chicks were helped out of the shell and the remaining eggs failed to hatch.

Lot C (check). Ten eggs were dipped in the corrosive sublimate solution and placed in the same incubator with Lots A and B. Seven of these eggs hatched, two pipped but the chicks died in the shell, one egg was infertile.

This experiment indicates that eggs containing the organism are less likely to hatch. Although these eggs were not infected under natural conditions the fact remains that eggs inoculated in the same manner with a sterile fluid hatched. So that the failure to hatch of the eggs inoculated with *Bact. pullorum* cannot be attributed to mechanical injury due to the injection. Later it will be shown that in the natural course of the disease, it is the yolk that first becomes infected.

Experiment 5. July 2, 1910. Testing the pathogenesis of *Bact. pullorum* in adult fowls.

Hen No. A 1034. This fowl received a hypodermic injection of 1.5 cc. of a forty-eight hour bouillon culture of *Bact. pullorum* in the muscles of the breast. She showed marked depression two days after the injection and appeared sick for three or four days but rapidly regained her normal health.

Hen A 1097. This fowl received 1.5 cc. of a forty-eight hour bouillon culture of *Bact. pullorum* in the wing vein. Two days after the inoculation marked depression was noticed. This condition lasted for three or four days. The bird then recovered and soon was back to her normal state of health.

Hens No. 1667 and No. 192 and a rooster were fed 200 cc. of a bouillon culture of *Bact. pullorum* in the drinking water. This was continued for three days. Symptoms did not develop in any of these fowls.

During this experiment the hens laid twenty-one eggs. These were incubated at 38 degrees C. and then examined bacteriologically for the presence of *Bact. pullorum*.

Technique. The eggs were placed in a rack the large end up. The shell was flamed with a bunsen burner and a hole cut in it with sterile scissors. Two loopfuls of the contents were removed from each egg and transferred to slant agar.

Egg No. 1 laid 7/28 pure cultures of *B. coli communis*.

Egg No. 2 laid 8/1 pure cultures unidentified organism.

Egg No. 3 laid 8/3 two colonies closely resembling *Bact. pullorum*.

Egg No. 4 laid 8/5 sterile.

Egg No. 5 laid 8/9 sterile.

Egg No. 6 laid 8/9 sterile.

Egg No. 7 laid 8/11 growth.

Egg No. 8 laid 8/13 growth.

Egg No. 9 laid 8/15 sterile.

Egg No. 10 laid 8/15 growth.

Egg No. 11 laid 8/18 growth.

Egg No. 12 laid 8/20 *colon bacillus*.

Egg No. 13 laid 8/20 organism resembling *Bact. pullorum* very closely, but on running through media it was found to differ in its fermentation properties.

Egg No. 14 laid 8/21 sterile.

Egg No. 15 laid 8/22 sterile.

Egg No. 16 laid 8/24 sterile.

Egg No. 17 laid 9/17 sterile.

Egg No. 18 laid 9/17 sterile.

Egg No. 19 laid 9/21 growth.

Egg No. 20 laid 9/24 growth.

Egg No. 21 laid 9/24 growth.

On October 4 and 5 the fowls were killed. All were examined very carefully for *Bact. pullorum*.

Hen A 1034. All organs appeared normal. Cultures were made from the heart, liver, lung, kidney, spleen, ovary and a yolk in the oviduct. All tubes remained sterile.

Hen A 1097. All organs appeared to be normal. Cultures were made from the internal organs and a yolk in the oviduct. In the ovary mixed cultures of *Bact. pullorum* were found and pure cultures of the same organism were obtained from the yolk. It differed somewhat from the stock cultures in that it produced a larger amount of gas in the glucose and the mannite bouillon. The original culture had the characteristic form of growth on slant agar.

A 1607. All organs including the ovary were normal in appearance and found to be sterile.

P 197. All organs were normal in appearance and sterile.

Rooster. All organs, including the testicles, were sterile and normal in appearance.

This experiment indicates that *Bact. pullorum* is not pathogenic for adult fowls. Although the organism was found in the ovary and an egg in the oviduct of a hen injected intravenously with the culture, it is not conclusive that it existed there as the result of the inoculation. The fowl may have previously been infected. Further experimentation on this point is necessary.

The finding of the bacterium in the ovary would account for the eggs containing the organism. Rettger examined a number of adult fowls and found they contained pathological ovaries from which cultures of *Bact. pullorum* were obtained.

Experiment 6. Infecting chicks and rearing the fowls that recovered from the disease.

July 19, 1910, forty chicks were each given orally by means of a pipette 0.15 cc. of a forty-eight hour bouillon culture of *Bact. pullorum*. Twenty of the forty chicks inoculated received the stock culture and twenty were inoculated with the organism isolated from an egg in Experiment 3. By August 10 thirty-four of the chicks had died of the disease, seventeen dying from infection with the stock culture and seventeen dying from infection with the egg culture. The six remaining chicks had shown symptoms of the disease but recovered and continued to grow. At first these were to have been killed December 1, but it was decided to keep these birds over until spring and breed from them then. Bacterial examination will be made of some of the eggs and the rest will be incubated and chicks hatched and the mortality will be noted. The ovaries and testicles of the parent birds will then be examined bacteriologically for the presence of *Bact. pullorum*.

So far the experiment has shown that the culture isolated from an infected egg is just as virulent as the stock culture. It also shows that certain young chicks overcome the infection.

Experiment 7. Owing to some of the older chicks resisting and overcoming the infection when inoculated artificially with pure cultures of *Bact. pullorum*, it was deemed necessary to determine at what age the chicks were most susceptible to infection and at what age they became immune.

Six pens were used and sixteen chicks were placed in each pen. The pens were numbered according to the day on which the chicks were infected. Each chick was inoculated orally by means of a pipette with 0.15 cc. of a forty-eight hour bouillon culture of *Bact. pullorum*.

Pen 1. October 20, 1910. The chicks in this pen were fed the culture when they had reached the age of twenty-four hours. At the end of three weeks nine of the sixteen chicks had died of the disease. The remainder were killed and bacterial examination revealed the organism in four of them.

Pen 2. October 21, 1910. The chicks in this pen were fed the culture when they had reached the age of forty-eight hours.

Chick No. 1 died October 25. Pure cultures of *Bact. pullorum* obtained from heart, liver and yolk.

Chick No. 2 died October 26. Pure cultures of *Bact. pullorum* obtained from liver and yolk, mixed from heart.

Chick No. 3 died November 4. Pure cultures of *Bact. pullorum* obtained from liver and yolk.

Chick No. 4 died November 6. Pure cultures of *Bact. pullorum* obtained from liver and yolk, mixed cultures from heart.

The remainder were killed November 10, twenty days after infection. Inoculations on slant agar were made from the heart, liver and yolk of each chick. *Bact. pullorum* was found in only one of these chicks. In this chick a pure culture was obtained from the heart and mixed cultures from the yolk and liver. Unabsorbed yolks were found in only two of the chicks that were killed.

Pen 3. October 22, 1910. The chicks in this pen were fed the cultures when they had reached the age of three days.

Chick No. 1 died October 23. Unable to find the organism in the heart, liver or yolk.

Chick No. 2 died November 8. Pure cultures obtained from the heart blood, liver and yolk. This chick was large and well developed and had not shown any symptoms of the disease before death. It was found dead in the morning.

At the end of one month the remaining chicks were well developed and strong. None of them showed symptoms of the disease.

Pen 4. October 23, 1910. The chicks in this pen were fed the culture when they reached the age of four days.

At the end of one month these chicks had not shown symptoms of the disease and no mortality had occurred.

Pen No. 5. October 24, 1910. The chicks in this pen were fed the culture when they had reached the age of five days. They all remained well.

Pen No. 6 (check). These chicks remained well.

This experiment indicates that chicks show the greatest susceptibility to infection during the first twenty-four hours of life. The chances of infection seem to decrease as the chick becomes older. The mortality of the chicks that were fed the culture at the age of twenty-four hours amounted, at the end of twenty-one days, to 56½ per cent. and 82 per cent. of them revealed the presence of the organism. They averaged fifty-two grams in weight at the end of ten days. The mortality of those fed the culture at the age of forty-eight hours amounted to 25 per cent. and 31 per cent. of them revealed the presence of the organism. The weight of these chicks was fifty-two grams at the end of ten days. Several of the chicks had shown symptoms of the disease but recovered from the infection.

In the chicks fed the culture at the age of three days the mortality was only 6¼ per cent. from the disease. Their average weight was 58.6 grams, a gain of 6.6 grams per chick over Pens 1 and 2. None of those fed the culture at the age of four and five days developed the disease. Their average weight and that of those in the check pen was fifty-nine grams, showing plainly that the ingested organism did not retard the development of the chicks in these pens.

At the conclusion of the preceding experiment two three-day old chicks were inoculated subcutaneously with 0.5 cc. of a forty-eight hour bouillon culture of *Bact. pullorum*. The first one died at the end of ten days. Bacteriological examination disclosed the organism in pure cultures in the heart blood, liver and yolk. The other chick died seventeen days after infection. The organism was recovered in pure cultures from the heart blood and liver, but mixed cultures were obtained from the yolk.

Two chicks four days of age were injected subcutaneously with 0.5 cc. of a forty-eight hour bouillon culture of *Bact. pullorum*. Two days after infection the chicks showed marked depression. They recovered slowly and at the end of one month seemed to be well although their growth was retarded.

Experiment 8. This experiment consisted of infecting the shells of eggs with pure cultures of *Bact. pullorum*. The large end of the egg was smeared with a sterile swab dipped in a twenty-four hour bouillon culture. The eggs were then permitted to dry and incubated full time. This experiment was tried earlier in the summer but the eggs failed to hatch. The second trial gave the following results:

Pen No. 1 (twenty eggs smeared with the bouillon culture). November 15. Eleven chicks hatched.

Chick No. 1 died November 17. Inoculations on slant agar from the heart blood, liver and yolk remained sterile.

None of the remaining chicks revealed symptoms of the disease and at the end of one month none of them had died.

Pen No. 2. Check (twenty eggs placed in the incubator without being smeared). Twelve of these eggs hatched. None of the chicks died during the first month.

This experiment indicates that infection of chicks does not take place from *Bact. pullorum* which may have been present on the outside of the shell at the time of placing the eggs in the incubator. Inoculations on slant agar from scrapings of the shells of these smeared eggs failed to reveal the presence of the organism. This would seem to indicate that the organism would not live three weeks in an incubator having an average temperature of 103° F.

Sterile swabs were sent to poultrymen whose young stock were infected with the disease. These swabs were smeared around the inside of the incubators and returned. Slant agar tubes were then streaked with the swab. None of the cultures revealed the presence of *Bact. pullorum*.

PREVENTION.

Fatal septicemia or bacillary white diarrhea, like most of the infectious diseases, probably can be prevented and eradicated. The most important step is the prevention of the spread of the virus from the infected chicks to the healthy ones.

Experiments have shown that the disease is transmitted in three ways: (1) Through the egg. (2) By direct contact in the incubator with individuals that have acquired the disease through egg infection. (3) By day old chicks being placed in contaminated surroundings.

It has previously been suggested that the greatest infection takes place within the incubator during and immediately after hatching. Although the probable number of eggs infected with *Bact. pullorum* is small, a chick hatched from an infected egg could infect a whole hatch. To guard against this it would be well just before the eggs hatch to place them in a pedigree tray. This tray consists of a metal box so constructed that it is divided into a large number of small compartments. Each compartment has a covering of wire. Not more than three eggs should be placed in a compartment. After hatching the chicks should be

left there until they are removed from the incubator. They can then be placed in a clean hover. Instead of placing the whole hatch of chicks together it would be better to divide the hover space by means of boards into as many pens as convenient. Not over ten chicks should be kept in a pen. It would be well to place the same chicks from the incubator compartment in the same pen. This segregation should be continued until the chicks have reached the age of four days. The boards can then be removed and the chicks permitted to run together. All chicks showing symptoms should be removed to a special pen. Those recovering from the disease should not be used for breeding purposes. The chicks that did not show symptoms of the disease can be used as breeders in the spring. It is recommended that the old fowls be marketed or kept separate from the young stock, as they may be spreading the infection.

Too much stress cannot be placed on the thorough disinfection of the hovers and brooders and the burning of the used litter. For disinfecting purposes the following is recommended: one part crude carbolic acid, one part commercial sulphuric acid and forty parts of water. The acids should be mixed in a wooden vessel and the water added slowly. The hovers, floors and runways should be thoroughly sprayed with this solution. Scalding of the feeding and drinking utensils is recommended. Another point to be borne in mind is the indiscriminate purchasing of eggs for hatching and of day old chicks. Several serious outbreaks of the disease have come to our notice caused by the hatching of eggs from infected sources.

Trap nesting and the pedigreeing of chicks will enable the poultryman to locate the parent birds that are spreading the disease.

CONCLUSIONS.

After careful experimentation the following conclusions seem to be justifiable:

(1) *Bact. pullorum* causes fatal septicemia or bacillary white diarrhea in young chickens.

(2) Chicks are most susceptible to infection during the first twenty-four hours of life. They are less susceptible after they have reached the age of forty-eight hours. They seem to be immune from infection after the fourth day of life.

(3) Adult fowls are not susceptible to the disease.

(3) *Bact. pullorum* was found in the egg, although eggs inoculated artificially with the organism failed to hatch.

(5) The disease seems to be introduced into new districts by the indiscriminate buying of eggs for hatching and the purchase of day old chicks from infected poultry farms.

(6) The prevention of the disease seems to consist in the segregation of the chicks until they have reached the age of four days, and careful disinfection of the incubators, brooders, runways, and feeding and drinking utensils.

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